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On the Mechanism of Developed Paralyses in Cold-Blooded Animals (Frogs) in Experimental Botulism

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During investigations performed on warm-blooded animals (1,2,3) we determined that botulinal toxin impairs, in the first place, the motor neurons of the anterior horn of the spinal cord and, then, one can notice, only after several days following the intoxication, the impairments of neuromuscular terminal apparatuses. At the same time, the afferent part of the spinal reflex arch remains unaffected.

We found only partial reports (4, 5, 6, 7) in the literature dealing with the question of the mechanism of botulinal toxin's effects on cold-blooded animals. Thus, we set the following goals in the present work:

1) To clarify the sequence of changes in functional properties of the spinal cord motor centers and of their conductors at various stages of botulinal intoxication;

2) To clarify whether botulinal toxin affects the afferent part of the reflex arch.

The experiments were performed on frogs (Rana temporaria) weighing 40 to 50 gm. One group of animals received in advance botulinal toxin type A (1 mice = Dlm = 0.00005 mg of dry toxin) administered into the spinal lymphatic sac in a quantity of 0.1 ml and in concentrations from $2 \cdot 10^{-3}$ to $5 \cdot 10^{-3}$ gm/ml; the other group received the toxin's injection into musculus gastrocnemius in a quantity of 0.1 ml, in dilutions from $1 \cdot 10^{-3}$ to $1 \cdot 10^{-4}$ gm/ml. The first method of administration of the toxin developed a general botulism picture, and the second one - a local picture (in a form of paralysis in one affected hindpaw). However, in some experiments. the local form of botulism changed to a general form after several days. The experiments were performed on animals with both forms of botulism on the first day and up to the seventh day following the inoculation. After decapitation of frogs, their spinal mount was securely fixed on dissecting stand with the dorsum upward, then we dissected off both sciatic nerves and also the tendons of tibial muscles. We connected the latter with Engelman's myographs.

About 10 to 12 minutes after the preparation, we performed the neurotomy of one of the sciatic nerves, then we separated the central end of the tibial nerve and arranged it on platinum electrodes connected with the outlet of the GRAKH-1 stimulator that enabled us to change the frequency, the intensity and the duration of stimulating impulses. The stimulation of the indicated nerves caused a contraction of the opposite intact side of the gastrocnemius muscle. Thus, indirectly to the optimum and pessimum of the stimulation frequency, we investigated in these experiments the lability

of the spinal motor centers. Later, with the stimulation of the peripheral end of the neurotomized nerve, we investigated by analogous method the lability of the myoneural connections and, finally, muscles. Along with the lability, we also investigated the rheobase, as well as the chronaxy of the sciatic nerve, and of the gastrocnemius muscle.

We performed identical experiments on normal frogs for control purposes.

In 25 experiments with spinal preparations of uninfected frogs, contractions of the gastrocnemius muscle appeared with the stimulation of the central end of the neurotomized tibial nerve on the opposite side, when frequencies from 1 to 80 cps were used. As a rule, with the frequencies of the aforementioned magnitude, we observed pessimal contractions of the investigated muscle. Thus, according to the optimum and pessimum of the stimulation frequency, the lability of motor centers of the spinal cord ranged in control experiments about 80 cps. The gastrocnemius muscle contracted on the frequencies from 350 to 400 cps during stimulation of the sciatic nerve in uninfected frogs; however, the optimum tetanus was observed with the stimulation of the nerve up to 60 cps, and the pessimum tetanus - from 90 cps up. The gastrocnemius muscle in direct stimulation responded to contractions on frequencies up to 230 cps. The rheobase of the tibial nerve varied from 0.2 to 0.6 v in separate experiments, however it remained within the range of 0.3 to 0.4 v in most experiments. The magnitude of the rheobase of the gastrocnemius muscle was 1 to 3 v, while the chronaxy varied

from 0.2 to 1.2 msec.

Figure 1 - Lability decrease in spinal motor centers on the side of inoculation with botulinal toxin after stimulation of the central end of the neurotomized tibial nerve on nonparalyzed side. Above - the myogram of the frontal tibial muscle of the paralyzed extremity; below - stimulation markings (stimulating current strength: 1.8 v); bottom - time markings 5 sec.

Figure 2 - Lability changes of peripheral motor tracts on a paralyzed side. Above - contraction records of the frontal tibial muscle during stimulation of the peroneal nerve in a paralyzed extremity; below - stimulation markings with indicated frequency (cps) of the stimulating current (current strength: 1.8 v); bottom - time markings 5 sec.

The basic experiments were carried out on 98 frogs. In 30 experiments the toxin was administered into the spinal lymphatic sac and in 68 experiments - by intramuscular method. A series of successive stages of developed injuries in the nervous system were

detected with local and general forms of botulism during investigation of functional conditions of various sections of the spinal reflex arches.

In the initial stages of intoxication (1st or 2d day after inoculation) we observed in 8 experiments with general forms of botulism and in 8 instances of local botulism that a considerable decrease occurred in lability of the spinal motor centers. In frogs with a local botulism this manifested itself only on a side of the administered toxin. As we see from figure 1, a contraction of the gastrocnemius muscle in these animals occurred only with low frequency (2 to 16 cps) stimulations administered to the central end of the neurotomized tibial nerve on the opposite side. We also noted a considerable fatigue of spinal neural centers, namely: a reflex response on the second stimulation could only be obtained after 10 to 15 minutes following a preceeding stimulation. In these experiments the rheobase, chronaxy and lability of the tibial nerve and of gastrocnemius muscle did not differ from those observed in control animals.

In the following 6 experiments with general form of botulism and in 9 experiments with local form of botulism (on the 2d or 3d day after inoculation), we could not record any contractions at all of the gastrocnemius muscle after stimulation of the central end of the tibial nerve, by any frequencies. However, the stimulation of the truncus of the tibial nerve has not produced any significant disorders in its functions, i.e. the magnitudes of the rheobase, chronaxy and lability appeared close to normal. The disorders appeared

only after a considerably high inoculation volume and they involved, in addition to spinal centers, also the peripheral motor conductors.

Figure 3, a - Recording of contractions of the frontal tibial nerve following direct stimulation on nonparalyzed side; b - recording of contractions of analogous muscle on the opposite and paralyzed side; below - markings of stimulation; bottom - time markings 5 sec.

Likewise, no reflector contractions of the gastrocnemius muscle were noticed. At the same time, changes appeared in functional characteristics of the tibial nerve: the rheobase increased to 7 v and above, the chronaxy lengthened and the lability showed a shifting tendency toward high frequencies. The functional characteristics of the gastrocnemius muscle showed no deviations from a normal state We observed similar changes in 6 experiments with a general form of botulism and in 14 experiments with a local form of botulism.

In the subsequent stage of intoxication, on the 2d to 4th day following the administration of toxin (in 3 experiments with a general form of botulism and in 19 with a local form of botulism), the injury of the tibial nerve was still expressed more distinctly:

usually, the rheobase and the chronaxy remained undetermined, even when the strength of stimulating current reached 100 v. The stimulation of the tibial nerve with the frequency of 30 cps and above caused contraction of the muscle in response to insignificant strength of the stimulating current: 1.8 v (see figure 2). Attention must be directed to a characteristic change in contractions of muscles: according to their form, they represented typical tonic contractions. However, according to comparison with a normal state, after direct stimulation of the inoculated gastrocnemius muscle, no important changes were noticed in the rheobase, chronaxy and lability.

In the very late stage, on the 4th to 7th day after inoculation (in 4 experiments with a general and in 5 experiments with a local form of botulism), we observed a complete absence of any effect following indirect stimulation of inoculated muscle at the time, when its direct stimulation caused a usual contraction. Figure 3 shows contraction curves after direct stimulation with various frequencies applied on gastrocnemius muscle infected with botulinal toxin and also the curves of muscle uninfected with botulism for comparison purposes. The infected gastrocnemius muscle, while entirely unresponsive to indirect stimulation, revealed on direct stimulation insignificant changes in the optimum and pessimum of the stimulation frequency.

In addition, we solved a question of injuries by toxin in the afferent sections of the spinal reflex arches. We administered botulinal toxin intramuscularly in doses that caused paralysis only in the inoculated extremity. During stimulation of the central end of

the neurotomized tibial nerve in the paralyzed extremity, reflex contractions in the gastrocnemius muscle always appeared on the side opposite to the nonparalyzed side; consequently, botulinal toxin does not affect the afferent sections of the spinal reflex arches.

Thus, the injury to the motor innervation of skeletal muscle in frogs is conditioned, first of all, by the toxin's effect on motor centers of the spinal cord. In this respect there is a complete conformity with the mechanism of the botulinal toxin's effect on warm-blooded animals. Evidently, disorders in functional characteristics of the peripheral motor nerves occur only after injury of the motor center at the time of its direct cooperation.

Conclusions

1. The administration of botulinal toxin type A in a dose of 0.1 in dilutions of $2 \cdot 10^{-3}$ gm/ml, or $5 \cdot 10^{-3}$ gm/ml into spinal lymphatic sac causes on the 2d or 3d day a development of a general form of botulism, and death on the third or fourth day.

The administration of botulinal toxin type A in a dose of 0.1 in dilutions of $1 \cdot 10^{-3}$ gm/ml, or $1 \cdot 10^{-5}$ gm/ml, causes a development of local botulism and paralysis of the affected extremity.

2. The development of paralyses, local and general, begins, in the first place, with the injurious effect of the toxin on pertinent motor centers of the spinal cord and, then, the harmful effect extends to the efferent part of the reflex arch.

3. The afferent part of the spinal reflex arches is not affected by botulinal toxin type A.

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Summary (copied)

The mechanism of the paralytic effect of botulinum toxin on the motor innervation of skeletal muscles was studied in frogs. It was established that paralysis of skeletal muscles begins from the injury of motor centers of the spinal cord. It was possible to reveal the affection of the peripheral neuromuscular apparatuses only after a considerable period of time following complete exclusion of the spinal nervous centers.